

25-hydroxyvitamin D, insulin-like growth factor 1 and metabolic syndrome at age 45y: a cross-sectional study in the 1958 British birth cohort

Elina Hyppönen¹, PhD, Barbara J Boucher², MD, FRCP, Diane J Berry¹, MSc, Chris Power¹, PhD

¹Centre for Paediatric Epidemiology and Biostatistics, UCL Institute of Child Health, 30 Guilford street, London, WC1N 1EH, UK.

²Centre for Diabetes and Metabolic Medicine, Institute of Cell and Molecular Science, Barts & the London, Queen Mary School of Medicine and Dentistry, 4 Newark St, London E1 2AT, UK

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Corresponding Author:

Dr Elina Hyppönen
Center for Paediatric Epidemiology and Biostatistics
Institute of Child Health
30 Guilford Street
London, WC1N 1EH.
e.hypponen@ich.ucl.ac.uk

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ABSTRACT

Objective: Hypovitaminosis D and reduced insulin-like growth factor-1 (IGF-1) are associated, individually, with metabolic syndrome. Physiologic interactions between vitamin D and IGF-1 are reported; this is the first study to investigate their combined associations with metabolic syndrome prevalence.

Research Design and Methods: Data on 25-hydroxyvitamin D [25(OH)D], IGF-1, and metabolic syndrome abnormalities (abdominal obesity, raised HbA1c, blood pressure and triglycerides and low HDL-cholesterol) were collected from 6810 British whites in the 1958 cohort, surveyed 2002-2004 (age 45y).

Results: IGF-1 concentrations increased with 25(OH)D up to ~75-85nmol/l, but not thereafter. Both 25(OH)D and IGF-1 were inversely associated with metabolic syndrome. There was an interaction between 25(OH)D and IGF-1 ($p=0.025$) on metabolic syndrome prevalence: IGF-1 was not significantly associated with metabolic syndrome among those with the lowest levels of 25(OH)D [$p>0.09$] whilst higher 25(OH)D was associated with metabolic syndrome at all IGF-1 concentrations ($p\leq 0.006$). Metabolic syndrome prevalence was lowest for participants with the highest concentrations of both 25(OH)D and IGF-1 [OR for highest vs. lowest third of both = 0.26 (0.17, 0.40), $p<0.0001$; adjusted for sex, month, hour, inactivity, alcohol consumption, smoking and social class]. 25(OH)D was associated with the prevalence of high HbA1c, blood pressure and triglycerides after adjustment for IGF-1, obesity and social and lifestyle variations ($p\leq 0.004$ for all comparisons).

Conclusions: Serum 25(OH)D is inversely associated with metabolic syndrome, while the inverse association with IGF-1 was found only among those without hypovitaminosis D. These results suggest that metabolic syndrome prevalence is the lowest when both 25(OH)D and IGF-1 are high.

KEY WORDS. vitamin D, 25-hydroxyvitamin D, insulin-like growth factor 1, metabolic syndrome, , obesity, cohort study, hypovitaminosis D, human

Hypovitaminosis D, reductions in circulating insulin-like growth factor [IGF-1] and in certain IGF-1 binding proteins (especially IGFBP-1) have been reported to be associated with metabolic syndrome and its individual components. Both vitamin D status and IGF-1 concentrations are reduced in obesity (1,2), however, for both factors, lower concentrations have been associated with disturbed glucose metabolism (3-8), high blood pressure (9-11), adverse lipid profiles (2,12,13), and cardiovascular disease (8,14,15) independent of body mass. The evidence for associations between vitamin D and IGF-1 axes with metabolic risk includes prospective studies, clinical trials and dose related effects, suggesting that associations may prove to be causal. Mechanisms by which vitamin D and IGF-1 axes may lead to human disease are, however, not fully understood and despite evidence for physiological interaction between these risk factors (16-18), little is known about their joint effects, and we can find no previous studies investigating their combined associations with metabolic syndrome.

Vitamin D is a hormone precursor, which before exerting its metabolic effects undergoes two successive hydroxylations. The first converts vitamin D to 25-hydroxyvitamin D [25(OH)D; serum concentration of 25(OH)D being an accepted indicator of vitamin D status] and the second converts 25(OH)D to the main active hormonal form, 1-25-dihydroxyvitamin D [1,25-(OH)₂D]. Hypovitaminosis D appears to act both through reductions in intracellular calcium and through the effects of 1,25-(OH)₂D on the regulation of various target genes [e.g. reduced insulin secretion through reduction in islet beta cell calcium (19), hypertension through lack of suppression of the renin gene (20), certain cancers by dysregulation of relevant target genes (21), and also through dysregulation of immune and anti-inflammatory responses (22)]. Mechanisms associating hypovitaminosis D with adverse lipid profiles (12,13) are less well understood.

There is evidence for interrelations between vitamin D and IGF-1 axes; for example,

anti-cancer effects of increased vitamin D availability include promotion of anti-proliferative effects on various tissues through increases in IGFBP-1,-3,-5 production and suppression of cell growth-promoting IGFBP-2 (23). The effects of hormonal vitamin D on the IGF-1 axis follow the targeting of ligand bound vitamin D receptor (VDR) complexes to vitamin D response elements (VDREs) in the promoter regions of IGFBP-1,-2-5 and -6 genes, most of which are known to actively respond to 1-25(OH)₂D (23). IGF-1 administration can reduce hyperglycaemia in man but can also increase circulating 1-25(OH)₂D (17). Thus, IGF-1 could exert effects partly through changes in vitamin D activation, while 1-25(OH)₂D may act in part through changes in IGF-1 axis regulation. These findings provide the basis for interactions between these axes as reported experimentally in bony tissues (24,25) and in observational studies on breast density (18) and adiposity related factors (16) in humans.

Hypovitaminosis D is a world-wide problem (26,27) as are the increasing prevalence of metabolic syndrome, type 2 diabetes, and cardiovascular disease (28). We have, therefore, examined the associations of both vitamin D status [serum 25(OH)D] and circulating IGF-1 with metabolic syndrome and its individual components in nearly 7000 participants in the 1958 British birth cohort at age 45y (29).

RESEARCH DESIGN AND METHODS

Participants are from the 1958 birth cohort, which included all births in England, Scotland, and Wales during one week in March 1958 (n=16,751) (30,31). Most recently, participants were contacted between September 2002 and April 2004, when the majority were 45y [range 44y (31.1%) to 46y (0.4%)]. The target population for the survey consisted of 11,971 individuals currently living in Britain. Seventy-eight percent (n=9349) of the participants filled in a questionnaire, of whom 7591 (81%) also provided blood samples for measurement of serum 25-hydroxyvitamin D and IGF-1 concentrations. The 1958 cohort is almost entirely a white population (98%) and for these

analyses 154 individuals of other ethnic groups were excluded. The main analyses for this study were carried out on 6810 whites with full information on 25(OH)D, IGF-1 and on all the features of the metabolic syndrome. The 45y biomedical survey was approved by the South-East Multi-Centre Research Ethics Committee and written consent for use of information in medical research studies was obtained from the participants.

Weight and standing height, at 45y, were measured without shoes and in light clothing by a trained nurse using standardized protocol and equipment; waist circumference was measured by the nurse midway between the costal margin and iliac crest. Blood pressure was measured in a seated position, after 5 minutes rest, using an Omron 705CP automated sphygmomanometer with a large cuff for participants with a mid-upper arm circumference ≥ 32 cm; the measurement was repeated three times, and blood pressure determined as the average of all successful measurements.

Venous blood samples were obtained without prior fasting and posted to the collaborating laboratory. Serum 25-hydroxyvitamin D (25(OH)D) concentrations were measured using an automated IDS OCTEIA ELISA with an Dade-Behring BEP2000 analyser, standardised according to the mean Vitamin D External Quality Assessment Scheme (DEQAS) (32). Serum IGF-1 concentration was measured using the Nichols Advantage IGF-1 chemiluminescence immunoassay referenced against WHO 1st International Reference Reagent 1988; Insulin Like Growth Factor 1 87/518. Glycosylated haemoglobin (HbA1c) was assayed using high performance liquid chromatography standardised to the Diabetes Control and Complications Trial (DCCT)(33). Triglycerides total and HDL cholesterol were measured by standard autoanalyser methodology.

Metabolic syndrome was defined using modified criteria of the Third Report of the National Cholesterol Education Program's Adult Treatment Panel (ATP III)(34), as abnormality of ≥ 3 of the following: abdominal obesity (waist circumference ≥ 102 cm in men, ≥ 88 cm in women), high HbA1c ($>7\%$ or known/self

reported type 2 diabetes)(33,35), high blood pressure (blood pressure $\geq 140/90$ mmHg or use of antihypertensive medication), low HDL cholesterol (<1.0 mmol/l for men, <1.3 mmol/l in women, or use of lipid modifying medications) and hypertriglyceridemia [serum triglycerides ≥ 2.3 mmol/l (36)].

Potential confounding factors include measurements as follows. Socio-economic position was assessed using the Registrar General's occupational classification categorised as I & II (managerial and professional), III non-manual, III manual, IV & V (manual unskilled) from data collected at birth and 42y (or 33y if data at 42y were missing). Individuals who were institutionalised, retired or long-term unemployed were classified separately. Information on frequency of physical activity was collected at age 42y (coded as " <2 -3 times/month", "once/wk", "2-3 times/week" or "4-7 times/week"). Time spent watching a television (TV) /using a computer (PC) daily was reported at age 45y [coded as " <1 hour", "1-2 hours", and " ≥ 3 hours"(29)]. Smoking was recorded as "", "ex-smoker", "1-19 cigarettes/day" or " ≥ 20 cigarettes/day" based on smoking history recorded at ages 23y, 33y and 42y. Frequency and amounts of alcohol consumption were reported at age 42y (coded as "not in the last month", " \leq once/month", "2-4 times/month", "2-3 times/week", " >4 times/week"); 'quantity' was converted to standard units and coded as "none", "1-2 units", "3-4 units", "5-6 units", "7-9 units" and " ≥ 10 units" per drinking session. Information on current region of residence was based on Government Office Regions divided into sections as follows: Southern (South-East, South-West, Greater London), Middle (East Anglia, Midlands, and Wales), England (North, North West, Yorkshire and Humber) and Scotland.

Statistical analysis. Natural log transformation was used for 25(OH)D and IGF-1 (to achieve normal distributions and for calculating geometric means). Variation in continuous (log transformed) outcomes were evaluated by linear regression, and in dichotomous outcomes by logistic regression. P-values are from log

likelihood ratio tests (LRT); test for trend was preferred where appropriate.

Dichotomous indicators were created for components of metabolic syndrome (i.e. abdominal obesity, high HbA1c, high blood pressure, low HDL and high triglycerides) and these were used to create the defined indicator for the syndrome [described above]. The main outcome measures in our study are the *prevalence* of metabolic syndrome and the *prevalence* of its components, which hereafter we refer to as the metabolic syndrome or as the respective component.

The main analyses were conducted by logistic regression both for the metabolic syndrome and separately for its individual components, adjusting all models for gender, month and hour (for metabolic syndrome and high triglycerides) of measurement. Initial analyses included graphical examination of data and evaluation of curvature for 25(OH)D and IGF-1 through inclusion of quadratic terms. 25(OH)D and IGF-1 were categorized in thirds (or log transformed) for analysis, and single term and mutually adjusted models fitted. Interaction between 25(OH)D and IGF-1 was tested by including an interaction term of continuous log transformed indicators to the mutually adjusted model [i.e. $\log 25(\text{OH})\text{D} * \log \text{IGF}1$]. Due to the observed evidence for a significant interaction between $\log 25(\text{OH})\text{D}$ and $\log \text{IGF}-1$ on their association with metabolic syndrome ($p < 0.05$), we also present data from repeated analyses using a combined indicator for thirds of both 25(OH)D and IGF-1, thereby dividing participants into nine groups. In these analyses participants in the lowest thirds of both 25(OH)D and IGF-1 are the reference group. Further tests for interaction were done to evaluate possible effect modification by gender and obesity on the association of $\log 25(\text{OH})\text{D}$ and $\log \text{IGF}-1$, both treated as continuous variables, on the outcome (i.e. on metabolic syndrome and its components) and stratified analyses performed as warranted. The main modeling for metabolic syndrome was done in four stages, (1) starting with simple associations with 25(OH)D or IGF-1, (2) using mutual adjustment for both 25(OH)D and IGF-1, (3) with additional adjustment for demographic,

lifestyle and social factors and (4) with further adjustment for adiposity (BMI and waist circumference) for the other components of metabolic syndrome.

A fuller range of potential demographic, lifestyle and social confounders was investigated; birthweight, family history of diabetes, smoking, frequency and quantity of alcohol consumption, geographical location, frequency of physical activity, time spent watching TV or using PC, and social class at birth and at age 42 years. Adjustments for birthweight, quantity of alcohol, and geographical location were not included in final models as they did not affect the model fit and were not statistically significant (LRT $p > 0.05$). All analyses were carried out using STATA, version 9.

RESULTS

At age 45y, 9.6% ($n=315$) of men and 8.1% ($n=284$) of women had metabolic syndrome (Table 1). Amongst the individual components of metabolic syndrome, men were more likely to be classified as having disturbed glucose metabolism (2.5% vs. 1.5%, $p=0.005$), high blood pressure (32.9% vs 15.3%, $p < 0.001$) and high triglycerides (35.8% vs. 14.1%, $p < 0.001$), whilst the prevalence of low HDL-C and of abdominal obesity was lower in men than in women (4.4% vs. 18.9%; $p < 0.001$ and 29.7% vs. 34.5%; $p < 0.001$) respectively. Abdominal obesity was the most common feature of metabolic syndrome being present in 94.6% of men and 95.8% of women classified with the syndrome

Association between 25(OH)D and IGF-1.

Lifestyle and social indicators which were associated with the prevalence of metabolic syndrome tended to be associated also with concentrations of both 25(OH)D and IGF-1 (Table 1). There was a positive association between 25(OH)D and IGF-1, with a linear increase in IGF-1 until 25(OH)D concentrations reached ~ 75-85 nmol/l after which this effect reached a plateau (Figure 1). Adjustment for lifestyle and social factors [listed in Table 1] somewhat attenuated the association between 25(OH)D and IGF-1 and, although further adjustment for waist circumference and BMI

increased this attenuation effect, a clear independent association of these two variables persisted.

Prevalence of metabolic syndrome by 25(OH)D and IGF-1. Both 25(OH)D and IGF-1 were inversely associated with the prevalence of metabolic syndrome (Table 2). Mutual adjustment had only a small influence; however there was evidence for interaction between 25(OH)D and IGF-1 on their association with metabolic syndrome (LRT interaction $p=0.025$). As shown in Figure 2, greater IGF-1 was not significantly associated with the metabolic syndrome among participants with the lowest 25(OH)D concentrations [OR 0.90 (0.66, 1.20), $p=0.49$ and 0.77 (0.56, 1.05), $p=0.10$ for the middle and the highest third in IGF-1 compared to the lowest, respectively], whilst greater 25(OH)D was associated with a lower prevalence even among participants in the lowest third of IGF-1 [adjusted OR 0.64 (0.47, 0.88), $p=0.006$ and 0.47 (0.33, 0.68), $p<0.0001$ for the middle and the highest compared to the lowest third of 25(OH)D, respectively]. Prevalence of metabolic syndrome was the lowest in participants with the highest concentrations of both 25(OH)D and IGF-1 [OR 0.26 (0.17, 0.40), $p<0.0001$ for highest vs. lowest third in both, Figure 2].

Components of metabolic syndrome by increases in 25(OH)D and IGF-1. All the components used to define metabolic syndrome (abdominal obesity, high HbA1c, high blood pressure, low HDL-C and high triglycerides) were significantly associated with 25(OH)D in unadjusted analyses (Table 3). Associations between IGF-1 and components of metabolic syndrome were somewhat weaker than those observed for 25(OH)D, and for abdominal obesity and high serum triglycerides associations with IGF-1 were observed only in women (LRT interaction $p<0.0001$ for both comparisons). There was no evidence of a gender interaction with 25(OH)D in relation to any metabolic syndrome component (LRT interaction $p\geq 0.2$ for all comparisons). Mutual adjustment for 25(OH)D and IGF-1 had little influence on the associations between 25(OH)D and any of the components, whilst associations for IGF-1 were more strongly affected (the association with low

HDL-C was fully explained). Associations for 25(OH)D with abdominal obesity, HbA1c, high blood pressure and high triglycerides, but not with low HDL-C, remained strong after full adjustment for IGF-1, lifestyle and social factors, and obesity (Table 3). Associations for IGF-1 with abdominal obesity and high triglycerides persisted among women in fully adjusted models, while borderline associations with HbA1c and high blood pressure were observed for both genders. There was no evidence for interaction between 25(OH)D and IGF-1 in relation to any of the individual components of metabolic syndrome (LRT interaction $p\geq 0.08$ for all). Associations for 25(OH)D with all components of the metabolic syndrome were similar for obese and non-obese participants (LRT interaction $p>0.19$ for all comparisons); whereas associations between IGF-1 and high triglycerides were seen in obese [lifestyle adjusted OR 0.84 (0.67, 1.07) and 0.70 (0.55, 0.89), $p=0.0003$] but not in non-obese participants [1.07 (0.87, 1.3) and 1.2 (0.99, 1.5), $p=0.30$; LRT interaction $p=0.0006$]. There were no interactions by obesity in the association of IGF-1 with any other component (LRT interaction $p>0.13$ for all comparisons).

DISCUSSION

These findings from the 1958 British birth cohort (aged 45years) confirm inverse associations for both 25(OH)D and IGF-1 with the metabolic syndrome (3,8,14). In addition, examination of the interactions between these factors further suggests that the associated reductions in the prevalence of metabolic syndrome may be greatest when levels of both 25(OH)D and IGF-1 are high. 25(OH)D concentration was inversely associated with metabolic syndrome regardless of IGF-1 concentration, while no significant variation in metabolic syndrome by IGF-1 concentrations was observed if 25(OH)D concentrations were low. Interestingly, these findings are in accordance with previous observations on mammographic breast density and cancer risk (18), suggesting that the metabolic efficacy of IGF-1 in various tissues may vary according to the individual's vitamin D status.

Metabolic interaction has been reported between the vitamin D and IGF-1 axes experimentally with evidence to show that IGF-1 exerts some effects through changes in vitamin D activation while 1-25(OH)₂D in turn modulates the regulation of IGF-1 axis genes (16-18,37). We demonstrate a clear positive association between higher vitamin D status and increased circulating IGF-1 concentration, an association independent of important putative confounders such as lifestyle factors and adiposity. Circulating IGF-1 was observed to increase steeply up to, but not above, serum 25(OH)D concentrations of 75-85 nmol/l. The suggestion of a plateau for the association between 25(OH)D and IGF-1 at around 75-85 nmol/l is intriguing in the light of accumulating evidence suggesting that optimal adult 25(OH)D concentrations are likely to be ≥ 75 nmol/l (38). Support for this cut-off as a possible optimal concentration has been obtained in relation to various health outcomes (many bone-related) and indeed we have previously shown a steep decrease in average HbA1c concentrations with higher 25(OH)D concentrations up to 65 nmol/l with only small reductions at higher concentrations (6).

We have previously reported that the association of 25(OH)D with continuous HbA1c is more marked in obese as compared to normal weight individuals (6), while in the current study the association of 25(OH)D with high HbA1c ($>7\%$ or type 2 diabetes) did not vary by obesity. This may reflect lesser power in interaction analyses on dichotomous outcomes, or suggest that much of the previously reported interaction is explained by 25(OH)D influence on severity among participants who already have abnormal glucose metabolism. It is possible that in this relatively young population metabolic syndrome *per se* reflects the future risk of cardiovascular or diabetes morbidity better than any single abnormality and this could explain the lack of interaction for metabolic syndrome components in this cohort, despite an interaction for metabolic syndrome. An unexpected finding in our study was the association of IGF-1 with adiposity and high triglycerides in women but not in men. This gender difference may be related to hormonal differences since the women

in the present study were pre-menopausal, but the observation clearly deserves further study.

Methodological considerations. The main strength of the present study lies in the large, population-based sample of participants with information on vitamin D status [serum 25(OH)D], circulating IGF-1, and components of the metabolic syndrome. These data provide adequate power for detailed investigation of associations between these inter-related health indicators. Furthermore, given the extensive information available for the 1958 cohort, we were able to control for many recognized confounding factors, such as social circumstances, lifestyle variations and body size. Hence, it could be argued that since obesity is a strong determinant of vitamin D status (1,2,6) inclusion of obesity as a confounder may represent over-adjustment and that the true association of 25(OH)D with individual components of metabolic syndrome may, therefore, have been under-estimated. However, due to the observational cross-sectional design of our study, we are not able to establish specific mechanisms, nor to establish causality, for these associations.

Our study was restricted to Caucasians and hence the study does not suffer from problems of population stratification. However, our findings cannot be extrapolated to non-white ethnic groups, given the reported heterogeneity by ethnic origin reported for 25(OH)D influences on glucose and lipid metabolism (4). As recently described, some sample attrition has occurred during the survey and although the present sample remains broadly representative of the surviving cohort there is some under-representation of specific minority groups (39). An important limitation in our study is that, for practical reasons, fasting samples are not available. Hence, we were not able to adhere to recommended WHO criteria for metabolic syndrome, but modified it by using high HbA1c concentration to complement information on existing diabetes(33), and by using a cut-off for non-fasting triglyceride concentrations based on a screening procedure demonstrated to be effective in identifying diabetes (36,40). The association between IGF-1 and high triglycerides

was observed in obese but not in normal weight participants. As one potential explanation for this association, we have examined whether it was due to the use of non-fasting samples, with obesity interfering with the post-prandial increase in triglyceride concentrations. However, we found no statistical evidence suggesting that the association between time since last eating and serum triglycerides was affected by obesity ($p=0.34$) nor any evidence that the interaction between obesity and IGF-1 would have been affected by time since last eating ($p=0.71$). A further limitation of our study concerns the lack of information on IGF-BPs, which limits our ability to evaluate physiological effects of the IGF-1 axis, given that IGF-BPs play a major role in the determination of intracellular effects of IGF-1. Nonetheless, the associations found between circulating IGF-1 and metabolic syndrome, and importantly, the interaction found with vitamin D status [25(OH)D] suggests the interdependent involvement of both the IGF-1 and the vitamin D axes in the determination of metabolic syndrome. Further studies are required to establish the mechanisms by which these effects and their interactions are induced and randomized controlled trials are required to determine the extent to which prevention of hypovitaminosis D might ameliorate the risk of metabolic syndrome.

To conclude, the observed reductions in the prevalence of metabolic syndrome with higher vitamin D status [measured by serum 25(OH)D] were greatest when circulating IGF-1 concentrations were also high, though clear reductions were observed irrespective of IGF-1 concentration. Our results highlight the importance of improving vitamin D status in the general population for the prevention of adverse long-term health risks that hypovitaminosis D may impose both in the UK and globally (27).

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REFERENCES

1. Wortsman,J, Matsuoka,LY, Chen,TC, Lu,Z, Holick,MF: Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr.* 72:690-693, 2000
2. Sandhu,MS, Gibson,JM, Heald,AH, Dunger,DB, Wareham,NJ: Association between insulin-like growth factor-I: insulin-like growth factor-binding protein-1 ratio and metabolic and anthropometric factors in men and women. *Cancer Epidemiol.Biomarkers Prev.* 13:166-170, 2004
3. Boucher,BJ: Inadequate vitamin D status: does it contribute to the disorders comprising syndrome 'X'? *Br.J Nutr.* 79:315-327, 1998
4. Scragg,R, Sowers,M, Bell,C: Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. *Diabetes Care* 27:2813-2818, 2004
5. Chiu,KC, Chu,A, Go,VL, Saad,MF: Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am.J.Clin.Nutr.* 79:820-825, 2004
6. Hyppönen,E, Power,C: Vitamin D status and glucose homeostasis in the 1958 British birth cohort: the role of obesity. *Diabetes Care* 29:2244-2246, 2006
7. Sandhu,MS, Heald,AH, Gibson,JM, Cruickshank,JK, Dunger,DB, Wareham,NJ: Circulating concentrations of insulin-like growth factor-I and development of glucose intolerance: a prospective observational study. *Lancet* 359:1740-1745, 2002
8. Juul,A: Serum levels of insulin-like growth factor I and its binding proteins in health and disease. *Growth Horm.IGF.Res.* 13:113-170, 2003
9. Lind,L, Hanni,A, Lithell,H, Hvarfner,A, Sorensen,OH, Ljunghall,S: Vitamin D is related to blood pressure and other cardiovascular risk factors in middle-aged men. *Am J Hypertens.* 8:894-901, 1995
10. Scragg,R, Sowers,M, Bell,C: Serum 25-hydroxyvitamin D, Ethnicity, and Blood Pressure in the Third National Health and Nutrition Examination Survey. *Am J Hypertens.* 20:713-719, 2007
11. Capoluongo,E, Pitocco,D, Lulli,P, Minucci,A, Santonocito,C, Manto,A, Di Stasio,E, Zaccardi,F, Zuppi,C, Ghirlanda,G, Ameglio,F: Inverse correlation between serum free IGF-I and IGFBP-3 levels and blood pressure in patients affected with type 1 diabetes. *Cytokine* 34:303-311, 2006
12. Auwerx,J, Bouillon,R, Kesteloot,H: Relation between 25-hydroxyvitamin D3, apolipoprotein A-I, and high density lipoprotein cholesterol. *Arterioscler.Thromb.* 12:671-674, 1992
13. John,WG, Noonan,K, Mannan,N, Boucher,BJ: Hypovitaminosis D is associated with reductions in serum apolipoprotein A-I but not with fasting lipids in British Bangladeshis. *Am J Clin Nutr.* 82:517-522, 2005
14. Zittermann,A, Schleithoff,SS, Koerfer,R: Putting cardiovascular disease and vitamin D insufficiency into perspective. *Br.J.Nutr.* 94:483-492, 2005
15. Juul,A, Scheike,T, Davidsen,M, Gyllenborg,J, Jorgensen,T: Low serum insulin-like growth factor I is associated with increased risk of ischemic heart disease: a population-based case-control study. *Circulation* 106:939-944, 2002
16. Gomez,JM, Maravall,FJ, Gomez,N, Navarro,MA, Casamitjana,R, Soler,J: Relationship between 25-(OH) D3, the IGF-I system, leptin, anthropometric and body composition variables in a healthy, randomly selected population. *Horm.Metab Res.* 36:48-53, 2004
17. Gomez,JM: The role of insulin-like growth factor I components in the regulation of vitamin D. *Curr.Pharm.Biotechnol.* 7:125-132, 2006
18. Diorio,C, Berube,S, Byrne,C, Masse,B, Hebert-Croteau,N, Yaffe,M, Cote,G, Pollak,M, Brisson,J: Influence of insulin-like growth factors on the strength of the relation of vitamin D and calcium intakes to mammographic breast density. *Cancer Res.* 66:588-597, 2006

19. Davidson,HW, Rhodes,CJ, Hutton,JC: Intraorganellar calcium and pH control proinsulin cleavage in the pancreatic beta cell via two distinct site-specific endopeptidases. *Nature* 333:93-96, 1988
20. Li,YC, Kong,J, Wei,M, Chen,ZF, Liu,SQ, Cao,LP: 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J.Clin.Invest* 110:229-238, 2002
21. Garland,CF, Garland,FC, Gorham,ED, Lipkin,M, Newmark,H, Mohr,SB, Holick,MF: The role of vitamin D in cancer prevention. *Am.J.Public Health* 96:252-261, 2006
22. Peterlik,M, Cross,HS: Dysfunction of the vitamin D endocrine system as common cause for multiple malignant and other chronic diseases. *Anticancer Res.* 26:2581-2588, 2006
23. Matilainen,M, Malinen,M, Saavalainen,K, Carlberg,C: Regulation of multiple insulin-like growth factor binding protein genes by 1alpha,25-dihydroxyvitamin D3. *Nucleic Acids Res.* 33:5521-5532, 2005
24. Klaus,G, Weber,L, Rodriguez,J, Fernandez,P, Klein,T, Grulich-Henn,J, Hugel,U, Ritz,E, Mehls,P: Interaction of IGF-I and 1 alpha, 25(OH)2D3 on receptor expression and growth stimulation in rat growth plate chondrocytes. *Kidney Int.* 53:1152-1161, 1998
25. Kurose,H, Yamaoka,K, Okada,S, Nakajima,S, Seino,Y: 1,25-Dihydroxyvitamin D3 [1,25-(OH)2D3] increases insulin-like growth factor I (IGF-I) receptors in clonal osteoblastic cells. Study on interaction of IGF-I and 1,25-(OH)2D3. *Endocrinology* 126:2088-2094, 1990
26. Calvo,MS, Whiting,SJ, Barton,CN: Vitamin D intake: a global perspective of current status. *J.Nutr.* 135:310-316, 2005
27. Holick,MF: Vitamin D deficiency. *N.Engl.J Med.* 357:266-281, 2007
28. Eckel,RH, Grundy,SM, Zimmet,PZ: The metabolic syndrome. *Lancet* 365:1415-1428, 2005
29. Hyppönen,E, Power,C: Hypovitaminosis D in British adults at age 45y: nationwide cohort study on dietary and lifestyle predictors. *Am.J.Clin.Nutr.* 85:860, 2007
30. Butler NR, Bonham DG: Perinatal Mortality. Edinburgh, Livingstone, 1963,
31. Power,C, Elliott,J: Cohort profile: 1958 British birth cohort (National Child Development Study). *Int.J.Epidemiol.* 35:34-41, 2006
32. Hyppönen,E, Turner,S, Cumberland,P, Power,C, Gibb,I: Serum 25-hydroxyvitamin D measurement in a large population survey with statistical harmonization of assay variation to an international standard. *J.Clin.Endocrinol.Metab.* In press: 2007
33. Thomas,C, Hyppönen,E, Power,C: Diabetes risk in British adults in mid life: a national prevalence study of glycated haemoglobin. *Diabet.Med.* 24:317-321, 2007
34. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 285:2486-2497, 2001
35. Peters,AL, Davidson,MB, Schriger,DL, Hasselblad,V: A clinical approach for the diagnosis of diabetes mellitus: an analysis using glycosylated hemoglobin levels. Meta-analysis Research Group on the Diagnosis of Diabetes Using Glycated Hemoglobin Levels. *JAMA* 276:1246-1252, 1996
36. Wannamethee,SG, Shaper,AG, Durrington,PN, Perry,IJ: Hypertension, serum insulin, obesity and the metabolic syndrome. *J Hum.Hypertens.* 12:735-741, 1998
37. Capoluongo,E, Zuppi,C, Ameglio,F: IGF-1 system, Vitamin D and blood pressure relationships. *Cytokine* 37:183-184, 2007
38. Dawson-Hughes,B, Heaney,RP, Holick,MF, Lips,P, Meunier,PJ, Vieth,R: Estimates of optimal vitamin D status. *Osteoporos.Int.* 16:713-716, 2005
39. Atherton,K, Fuller,E, Shepherd,P, Strachan,D, Power,C: Loss and representativeness in a biomedical survey at age 45 years: 1958 British birth cohort. *J.Epidemiol.Community.Health.* in press: 2007

40. Lidfeldt,J, Nerbrand,C, Samsioe,G, Schersten,B, Agardh,CD: A screening procedure detecting high-yield candidates for OGTT. The Women's Health in the Lund Area (WHILA) study: a population based study of middle-aged Swedish women. *Eur.J Epidemiol.* 17:943-951, 2001

TABLE 1. 25-hydroxyvitamin D, insulin like growth factor 1 and prevalence of metabolic syndrome by sex, BMI, lifestyle and social characteristics in the 1958 British birth cohort (N=6810).

	Number (%)	25-hydroxyvitamin D (nmol/l)	IGF-1 (nmol/l)	Metabolic syndrome
		Geometric mean	Geometric mean	% (n)
Sex				
Men	3297 (48.4)	53.8	18.0	9.6 (315)
Women	3513 (51.6)	51.5	17.9	8.1 (284)
		p < 0.001	p = 0.51	p = 0.03
Body mass index				
<18.5	37 (0.5)	39.4	15.8	2.7 (1)
18.5 -24.9	2448 (36.0)	54.8	18.2	0.7 (18)
25-29.9	2808 (41.2)	53.9	18.2	6.3 (178)
30-34.9	1087 (16.0)	47.9	17.4	22.3 (242)
≥35	430 (6.3)	42.7	16.0	37.2 (160)
		p < 0.001	p < 0.001	p < 0.001
Physical activity				
<2 to 3 times per month	1748 (25.7)	49.6	17.5	11.6 (251)
1 per wk	2159 (31.7)	52.9	18.2	7.8 (97)
2 to 3 time per week	1252 (18.4)	56.0	18.4	7.7 (111)
> 3 times per week	1438 (21.1)	53.6	18.0	7.2 (126)
Unknown	213 (3.1)	51.1	17.6	6.6 (14)
		p < 0.001	p = 0.002	p < 0.001
Smoking				
None	3196 (46.8)	54.0	18.1	8.0 (255)
Ex-smoker	1850 (27.2)	54.9	18.0	8.8 (164)
1-19 per day	788 (11.6)	49.7	17.8	8.1 (64)
≥20 per day	755 (11.1)	45.0	17.3	13.3 (100)
Unknown	221 (3.3)	51.2	17.6	7.2 (16)
		p < 0.001	p < 0.001	p = 0.001
Alcohol consumption				
Not in last month	414 (6.1)	45.4	17.5	14.3 (59)
< 1 of month	931 (13.7)	47.0	18.4	12.9 (120)
2-4 times per month	1465 (21.5)	53.2	18.6	9.0 (132)
2-3 times per week	2190 (32.2)	55.3	18.1	7.4 (162)
> 3 times per wk	1791 (26.2)	54.2	17.3	6.9 (123)
Unknown	19 (0.3)	49.5	17.4	15.8 (3)
		p < 0.001	p < 0.001	p < 0.001
Family social class (1958)				
I & II (high)	1318 (19.3)	52.7	18.6	5.8 (77)
III non-manual	686 (10.1)	54.9	18.2	7.3 (50)
III manual	3286 (48.3)	53.1	17.8	9.2 (301)
IV & IV	1325 (19.4)	50.8	17.6	11.6 (154)
Other~	195 (2.9)	48.7	17.3	8.7 (17)
		p = 0.007	p < 0.001	p < 0.001
Adult social class (2000)				
I & II (high)	2779 (40.8)	53.3	18.4	7.9 (220)
III non-manual	1432 (21.0)	52.7	18.0	6.9 (99)
III manual	1262 (18.5)	53.5	17.6	10.9 (138)
IV & IV	1065 (15.7)	50.8	17.2	10.3 (110)
Other*	272 (4.0)	48.3	17.9	11.8 (32)
		p = 0.004	p < 0.001	p < 0.001

P-values from linear regression for analyses on log 25(OH)D and log IGF-1, and from a Chi-squared test for analyses on metabolic syndrome. IGF-1 and metabolic syndrome adjusted for sex; 25(OH)D adjusted for sex and season.

* Includes cohort members who are institutionalised, retired, unemployed and other

TABLE 2. Single and joint associations of 25-hydroxyvitamin D and insulin-like growth factor 1 with the metabolic syndrome in the 1958 British birth cohort (N=6810).

	Separate models* OR (95%CI)	Mutually adjusted† OR (95%CI)	Interaction‡ OR (95%CI)
25-hydroxyvitamin D			
<u>Lowest third</u> (9-45 nmol/l)	<u>Reference</u>	<u>Reference</u>	-
Middle third (46-67 nmol/l)	0.56 (0.46, 0.69)	0.58 (0.48, 0.72)	
Highest third (68-231 nmol/l)	0.31 (0.24, 0.39)	0.33 (0.26, 0.42)	
Per log unit increase§	0.31 (0.25, 0.38)	0.33 (0.27, 0.41)	2.01 (0.41, 9.95)
p-value	<0.0001	<0.0001	0.390
Insulin-like growth factor 1			
<u>Lowest third</u> (0-16 nmol/l)	<u>Reference</u>	<u>Reference</u>	-
Middle third (17-20 nmol/l)	0.76 (0.62, 0.93)	0.81 (0.66, 0.99)	
Highest third (21-72 nmol/l)	0.59 (0.48, 0.73)	0.67 (0.54, 0.83)	
Per log unit increase§	0.39 (0.30, 0.52)	0.48 (0.36, 0.63)	5.44 (0.63, 46.8)
p-value	<0.0001	<0.0001	0.123
log 25(OH)D * log IGF-1	-	-	0.52 (0.30, 0.92)
p-value			0.025

* Separate models; adjusted for sex, month and hour of measurement only.

† Mutually adjusted; sex, month and hour of measurement, 25(OH)D and IGF-1.

‡ Interaction: as mutually adjusted with interaction term (log 25(OH)D* log IGF-1).

§ 25(OH)D and IGF-1 in models as continuous, natural log transformed. Estimates can be interpreted as a three-fold increase in original scale, for example from 25nmol/l to 75nmol/l.

TABLE 3. Odds ratio of having each component of the metabolic syndrome by thirds of serum 25-hydroxyvitamin D and insulin-like growth factor 1 in the 1958 British birth cohort (N= 6293).

	25-hydroxyvitamin D			Insulin-like growth factor 1		
	OR (95% CI)*	Mutually adjusted OR (95%CI)†	Fully Adjusted OR (95%CI)§	OR (95% CI)*	Mutually adjusted OR (95%CI)†	Fully adjusted OR (95%CI)§
Abdominal obesity	<i>Men</i>					
<u>Lowest third</u>	<u>Reference</u>	<u>Reference</u>	<u>Reference</u>	<u>Reference</u>	<u>Reference</u>	<u>Reference</u>
Middle third	0.63 (0.55, 0.72)	0.64 (0.56, 0.74)	0.66 (0.58, 0.76)	0.87 (0.72, 1.1)	0.92 (0.76, 1.1)	0.92 (0.75, 1.1)
Highest third	0.39 (0.34, 0.45)	0.40 (0.35, 0.47)	0.44 (0.38, 0.51)	0.98 (0.81, 1.2)	1.06 (0.87, 1.3)	1.07 (0.88, 1.3)
Per log unit increase [§]	0.40 (0.35, 0.45)	0.41 (0.36, 0.47)	0.44 (0.38, 0.50)	0.97 (0.74, 1.3)	1.12 (0.85, 1.5)	1.13 (0.86, 1.5)
p-value	<0.0001	<0.0001	<0.0001	0.8	0.4	0.4
	<i>Women</i>					
<u>Lowest third</u>	-			<u>Reference</u>	<u>Reference</u>	<u>Reference</u>
Middle third				0.72 (0.60, 0.86)	0.74 (0.62, 0.88)	0.73 (0.60, 0.88)
Highest third				0.55 (0.46, 0.66)	0.60 (0.50, 0.72)	0.60 (0.50, 0.72)
Per log unit increase [§]				0.39 (0.31, 0.50)	0.44 (0.34, 0.56)	0.46 (0.36, 0.59)
p-value				<0.0001	<0.0001	<0.0001
High HbA1c						
<u>Lowest third</u>	<u>Reference</u>	<u>Reference</u>	<u>Reference</u>	<u>Reference</u>	<u>Reference</u>	<u>Reference</u>
Middle third	0.43 (0.28, 0.66)	0.45 (0.30, 0.70)	0.63 (0.40, 0.98)	0.67 (0.43, 1.0)	0.74 (0.47, 1.1)	0.79 (0.50, 1.3)
Highest third	0.14 (0.08, 0.26)	0.15 (0.08, 0.29)	0.30 (0.16, 0.57)	0.60 (0.38, 0.93)	0.71 (0.45, 1.1)	0.77 (0.48, 1.2)
Per log unit increase [§]	0.21 (0.14, 0.32)	0.23 (0.15, 0.35)	0.39 (0.24, 0.63)	0.32 (0.18, 0.55)	0.42 (0.24, 0.74)	0.52 (0.29, 0.97)
p-value	<0.0001	<0.0001	0.0001	0.0001	0.003	0.04
High blood pressure						
<u>Lowest third</u>	<u>Reference</u>	<u>Reference</u>	<u>Reference</u>	<u>Reference</u>	<u>Reference</u>	<u>Reference</u>
Middle third	0.72 (0.62, 0.83)	0.73 (0.63, 0.85)	0.80 (0.68, 0.94)	0.84 (0.73, 0.98)	0.87 (0.75, 1.00)	0.95 (0.81, 1.1)
Highest third	0.57 (0.49, 0.67)	0.59 (0.50, 0.70)	0.72 (0.61, 0.86)	0.72 (0.62, 0.83)	0.75 (0.65, 0.87)	0.85 (0.73, 0.99)
Per log unit increase [§]	0.59 (0.51, 0.68)	0.61 (0.53, 0.71)	0.74 (0.63, 0.87)	0.58 (0.48, 0.71)	0.63 (0.51, 0.77)	0.79 (0.64, 0.97)

p-value	<0.0001	<0.0001	0.0003	<0.0001	<0.0001	0.03
Low HDL						
Lowest third	<u>Reference</u>	<u>Reference</u>	<u>Reference</u>	<u>Reference</u>	<u>Reference</u>	<u>Reference</u>
Middle third	0.73 (0.60, 0.88)	0.74 (0.61, 0.89)	1.00 (0.81, 1.2)	0.86 (0.71, 1.1)	0.90 (0.74, 1.1)	0.96 (0.78, 1.19)
Highest third	0.47 (0.38, 0.59)	0.48 (0.38, 0.60)	0.86 (0.68, 1.1)	0.85 (0.70, 1.0)	0.92 (0.76, 1.1)	1.04 (0.85, 1.28)
Per log unit increase [§]	0.48(0.40, 0.58)	0.49(0.41, 0.59)	0.85 (0.69, 1.0)	0.72 (0.56, 0.94)	0.82 (0.63, 1.06)	1.05 (0.80, 1.39)
p-value	<0.0001	<0.0001	0.13	0.01	0.13	0.70
High Triglycerides						
Lowest third	<u>Reference</u>	<u>Reference</u>	<u>Reference</u>	<u>Reference</u>	<u>Reference</u>	<u>Reference</u>
Middle third	0.76 (0.66, 0.89)	0.77 (0.67, 0.90)	0.95 (0.81, 1.1)	1.04(0.86, 1.25)	1.07(0.89, 1.29)	1.06 (0.87, 1.30)
Highest third	0.48 (0.41, 0.57)	0.50 (0.42, 0.59)	0.74(0.61, 0.89)	1.03(0.86, 1.24)	1.08(0.90, 1.31)	1.05 (0.86, 1.28)
Per log unit increase [§]	0.51 (0.44, 0.60)	0.53 (0.45, 0.61)	0.80 (0.64, 0.99)	0.97 (0.75, 1.3)	1.06 (0.81, 1.38)	1.0 (0.75, 1.32)
p-value	<0.0001	<0.0001	0.004	0.81	0.67	0.98
<i>Men</i>						
Lowest third	-			<u>Reference</u>	<u>Reference</u>	<u>Reference</u>
Middle third				0.67(0.53, 0.86)	0.70(0.55, 0.90)	0.79 (0.60, 1.03)
Highest third				0.60(0.47, 0.77)	0.66(0.51, 0.84)	0.81 (0.62, 1.06)
Per log unit increase [§]				0.39(0.29, 0.54)	0.45 (0.32,0.62)	0.63 (0.44, 0.89)
p-value				<0.0001	<0.0001	0.01
<i>Women</i>						

There was no interaction between 25(OH)D and IGF-1 in relation to the individual components of metabolic syndrome (p>0.08 for all comparisons). Associations of IGF-1 with abdominal obesity and high triglycerides, stratified by gender, are shown because of significant effect modification (LRT interaction p<0.0001 for both comparisons).

* Adjusted for sex, month of measurement and hour of measurement for high triglycerides.

† Adjusted for sex, month of measurement, and 25(OH)D/IGF-1 as relevant. Analyses on high triglycerides further adjusted for the hour of measurement.

‡ Adjusted for sex, month of measurement, 25(OH)D/IGF-1 as relevant and social physical activity, smoking, alcohol consumption, and social class at birth and at age 42y. All but abdominal obesity also adjusted for body mass index and waist circumference. Analyses on high triglycerides further adjusted for the hour of measurement.

§ 25(OH)D and IGF-1 in models as continuous, log transformed. Estimates can be interpreted as a three-fold increase in original scale, for example from 25nmol/l to 75nmol/l.

FIGURE LEGENDS

Figure 1. Variation in insulin-like growth factor 1 (IGF-1) by serum 25-hydroxyvitamin D concentration. Model 1 (dashed, short): adjusted for month of measurement and sex. Model 2 (dashed, long): adjusted for month of measurement, sex, lifestyle and social indicators (physical activity, time spent watching TV/using PC, smoking, alcohol consumption and birth and adult social class). Model 3 (solid line): adjusted for month of measurement, sex, lifestyle indicators and adiposity (BMI and waist circumference). Values are coefficients from linear regression (reference <25nmol/l), 95% confidence intervals presented for model 3 by the shaded area.

Figure 2. Interaction between 25(OH)D and IGF-1 on the prevalence of metabolic syndrome; the 1958 British birth cohort (N= 6293). Values are Odds ratios as compared to the lowest thirds of both 25(OH)D and IGF-1 [95% confidence intervals presented by error bars] after adjustment for sex, month, hour, inactivity, alcohol consumption, smoking and social class at birth and at age 45y. Thirds in 25(OH) D: lowest 10-45nmol/l, middle 46-67 nmol/l, and highest 68-231 nmol/l; thirds in IGF-I: lowest 0-16 nmol/l, middle 17- 20 nmol/l, and highest 21-72nmol/l.

FIGURE 1

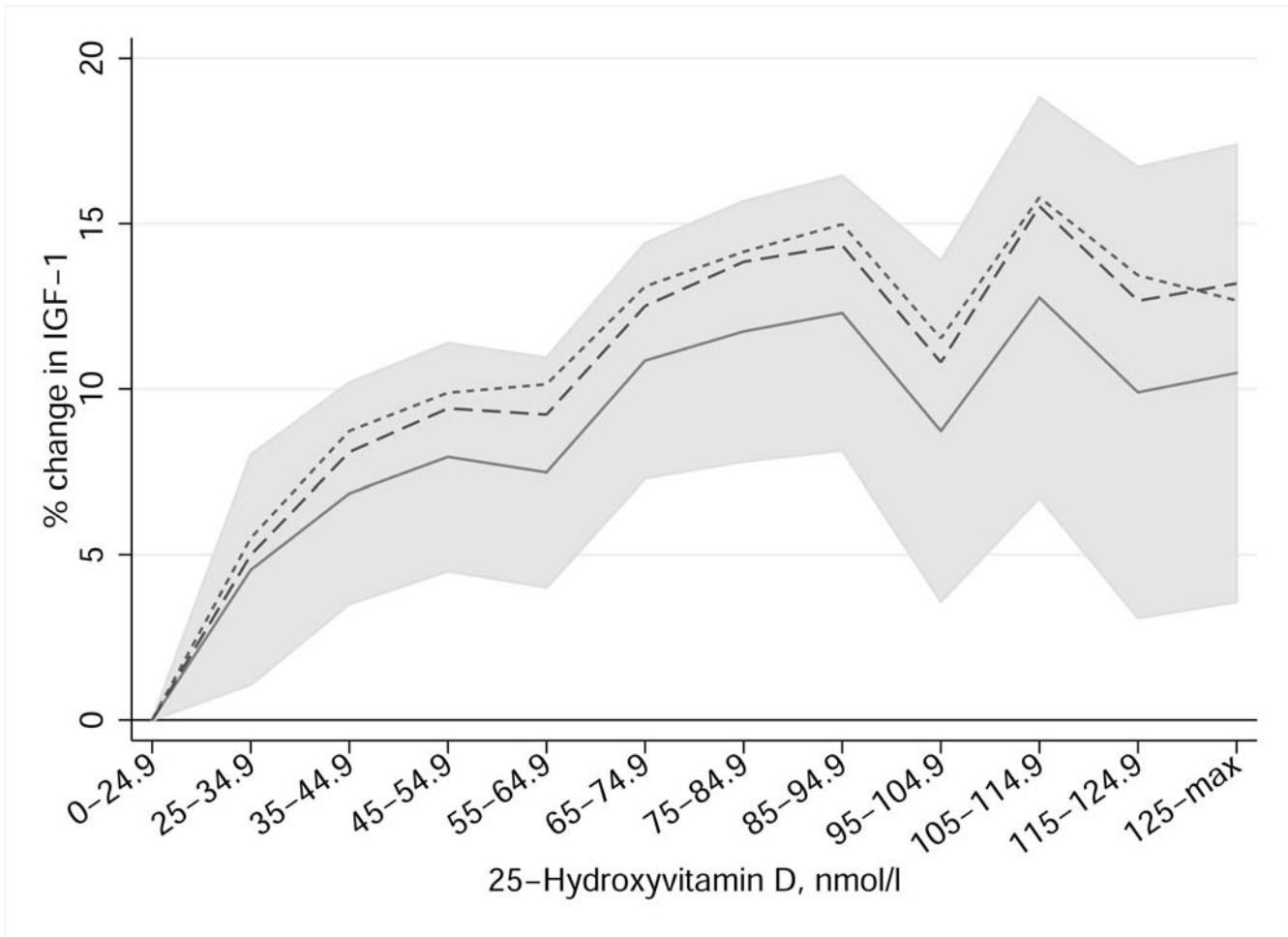


FIGURE 2

